

CLAIMS

1. A process of cloning a nucleic acid in a desired orientation comprising the steps of:
 - (a) obtaining a single stranded fragment of the nucleic acid;
 - 5 (b) ligating an oligonucleotide primer comprising at least one restriction enzyme recognition site to the 3' terminus of the fragment;
 - (c) producing a double-stranded nucleic acid using a primer complementary to the primer of step (b); and
 - (d) cloning the double-stranded nucleic acid into a desired vector.
- 10 2. The process of claim 1 wherein the nucleic acid is genomic DNA.
3. The process of claim 1 wherein the nucleic acid is cDNA.
- 15 4. The process of claim 1 wherein the nucleic acid is aRNA.
5. The process of claim 1 wherein step (b) further comprises ligating a specific primer to the 5' terminus of the fragment.
- 20 6. The process of claim 1 wherein step (c) further comprises using a primer complementary to the primer of claim 5.
7. The process of claim 5 wherein the primer comprises a restriction enzyme recognition site not present in the primer specific for the 3' terminus of the fragment.
- 25 8. The process of claim 1 wherein the ligation of step (b) is performed with T4 RNA ligase.
9. The process of claim 1 wherein step (c) is performed by polymerization with Klenow enzyme.
- 30 10. A process of cloning a nucleic acid in a desired orientation comprising the steps of:
 - (a) obtaining a single stranded fragment of the nucleic acid;
 - (b) ligating a double stranded adaptor comprising at least one restriction enzyme recognition site to each end of the fragment, wherein the adaptor ligated to the 5' terminus and the adaptor ligated to the 3' terminus differ in
 - 35 at least one restriction enzyme recognition site;

- (c) amplifying the fragment by PCR with a primer complementary to a portion of the adaptor of step (b) ligated to the 5' terminus and a primer complementary to a portion of the adaptor of step (b) ligated to the 3' terminus, to obtain a double-stranded nucleic acid; and
- 5 (d) cloning the double-stranded nucleic acid into a desired vector.
11. The process of claim 10 further wherein the adaptor ligated to the 3' terminus of the fragment in step (b) has a 5' nucleotide overhang.
- 10 12. The process of claim 11 further wherein the adaptor ligated to the 5' terminus of the fragment in step (b) has a 3' nucleotide overhang.
13. The process of claim 10 or 12 wherein the adaptors ligated to both ends of the fragment have nucleotide overhangs that differ from each other in sequence.
- 15 14. The process of claim 10 wherein the nucleic acid is genomic DNA.
15. The process of claim 10 wherein the nucleic acid is cDNA.
- 20 16. The process of claim 10 wherein the nucleic acid is aRNA.
17. The process of claim 10 wherein the ligation of step (b) is performed with T4 DNA ligase.
18. The process of claim 10 further comprising digesting the fragment of step (a) into smaller
25 fragments using a restriction enzyme.
19. The process of claim 18 wherein the adaptors used in step (b) further comprise the full or partial sequence of the restriction enzyme recognition site for the restriction enzyme used to digest the fragment of step (a) into smaller fragments.
- 30 20. A DNA library prepared according to the process of claim 1 or 10.
21. A kit for performing the process of claim 1 comprising:
- (a) a primer comprising at least one restriction enzyme recognition site that
35 can ligate to the 3' end of a nucleic acid; and

(b) T4 RNA ligase.

22. A kit for performing the process of claim 10 comprising:

- 5 (a) a double stranded nucleic acid adaptor comprising at least one restriction enzyme recognition site and capable of ligating to the 5' terminus of a single stranded nucleic acid;
- (b) a double stranded nucleic acid adaptor comprising at least one restriction enzyme recognition site and capable of ligating to the 3' terminus of a single stranded nucleic acid; and
- 10 (c) T4 DNA Ligase.

23. A process of digesting a single stranded nucleic acid with a restriction enzyme that digests double stranded nucleic acids comprising annealing at least one oligonucleotide comprising a sequence recognized by a restriction enzyme that digests double stranded nucleic acids to the
15 single stranded nucleic acid.

24. A double stranded nucleic acid adaptor comprising two oligonucleotides having a complementary region of 4 or more nucleotides and blocked 3' termini, wherein one oligonucleotide has a 3' overhang and lacks any phosphate on its 5' terminus.
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25. A double stranded nucleic acid adaptor comprising two oligonucleotides having a complementary region of 4 or more nucleotides and lacking any phosphate on the 5' termini, wherein one oligonucleotide has a 5' overhang and a blocked 3' terminus.

25 26. The adaptor of claim 24 or 25, wherein one of the oligonucleotides further comprises a single stranded region of 4 or more nucleotides.

27. The kit of claim 22 comprising the adaptors of claim 24 and claim 25.